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(FILE 'HOME' ENTERED AT 14:46:02 ON 02 JUN 2003)

FILE 'MEDLINE' ENTERED AT 14:46:10 ON 02 JUN 2003

L1 14297 S LEISHMANIA?  
L2 9896 S GM-CSF  
L3 30 S L1 AND L2  
L4 234336 S TRANSFECT? OR PLASMID? OR VECTOR?  
L5 1825 S L1 AND L4  
L6 1123 S L1 (P) L4  
L7 370 S L1 (3A) L4  
L8 207846 S TRANSFECT? OR PLASMID? OR HETEROLOGOUS  
L9 419 S L1 AND L8

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=> D BIB AB 198

L9 ANSWER 198 OF 419 MEDLINE  
AN 1998078996 MEDLINE  
DN 98078996 PubMed ID: 9419187  
TI Protection against **Leishmania** major challenge infection in mice vaccinated with live recombinant parasites expressing a cytotoxic gene.  
AU Muyombwe A; Olivier M; Harvie P; Bergeron M G; Ouellette M; Papadopoulou B  
CS Centre Hospitalier de l'Universite Laval et Departement de Microbiologie, Universite Laval, Quebec, Canada.  
SO JOURNAL OF INFECTIOUS DISEASES, (1998 Jan) 177 (1) 188-95.  
Journal code: 0413675. ISSN: 0022-1899.  
CY United States  
DT Journal; Article; (JOURNAL ARTICLE)  
LA English  
FS Abridged Index Medicus Journals; Priority Journals  
EM 199801  
ED Entered STN: 19980206  
Last Updated on STN: 19980206  
Entered Medline: 19980123  
AB A "suicide" system based on thymidine kinase-ganciclovir combination was developed and tested in a **Leishmania** major experimental model. Susceptible BALB/c mice were infected with L. major expressing the thymidine kinase gene of herpes simplex virus type 1 and treated for 2 consecutive weeks with 7.5 mg/kg/day ganciclovir at different times from the initial infection. Ganciclovir treatment at varying times after infection had different effects on the outcome of disease. A complete inhibition of intracellular parasites was obtained in mice treated 1 or 4 days after infection, whereas ganciclovir administration 2 weeks later resulted in the control of infection only when the drug was provided. Variable levels of protection, from partial to total, against challenge infection with virulent L. major were observed, depending on the timing of ganciclovir treatment. The thymidine kinase-ganciclovir approach represents an excellent experimental model to control **Leishmania** infection and to evaluate the immunologic response of the host.

INVENTOR

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L9 ANSWER 322 OF 419 MEDLINE  
AN 93267108 MEDLINE  
DN 93267108 PubMed ID: 8098724  
TI Transfected Leishmania expressing biologically active IFN-gamma.  
AU Tobin J F; Reiner S L; Hatam F; Zheng S; Leptak C L; Wirth D F; Locksley R M  
CS Department of Tropical Public Health, Harvard School of Public Health, Boston, MA 02115.  
NC AI 21365 (NIAID)  
AI 26918 (NIAID)  
SO JOURNAL OF IMMUNOLOGY, (1993 Jun 1) 150 (11) 5059-69.  
Journal code: 2985117R. ISSN: 0022-1767.  
CY United States  
DT Journal; Article; (JOURNAL ARTICLE)  
LA English  
FS Abridged Index Medicus Journals; Priority Journals  
EM 199306  
ED Entered STN: 19930702  
Last Updated on STN: 19950206  
Entered Medline: 19930622  
AB Infection of susceptible BALB/c mice with **Leishmania** major leads to progressive infection with the failure to expand and activate Th1 CD4+ T cells that elaborate IFN-gamma, a critically implicated cytokine for control of disease. We used the recently described capacity to express foreign genes in trypanosomatids to introduce into **Leishmania** the murine IFN-gamma gene on a drug-selectable **plasmid** under the constitutive control of intergenic tubulin sequences. Several clones of L. major were established and demonstrated to contain IFN-gamma DNA and IFN-gamma RNA that was appropriately trans-spliced with the **Leishmania**-specific leader sequence, and to secrete IFN-gamma into the media. The secreted IFN-gamma was biologically active as assessed by up-regulation of class II MHC Ag and induction of macrophage nitric oxide synthase activity in a macrophage cell line. Infection of nude mice with IFN-gamma-containing organisms resulted in significantly slower progression of disease as compared to infection with organisms containing the empty **plasmid**, suggesting that biologically important activation of infected macrophages might be occurring in vivo. Infection of genetically susceptible BALB/c mice, however, did not impede the expansion of Th2 cells and the inexorable progression of disease. Despite the demonstration of increased levels of IFN-gamma transcription in vivo, induction of nitric oxide synthase in macrophages and expression of Ly-6, and IFN-gamma-inducible Ag, on CD4+ lymphocytes could not be shown. In all cases, organisms recovered from tissue amastigotes contained the IFN-gamma **plasmid** and secreted active IFN-gamma. The data confirm earlier studies that IFN-gamma alone is not sufficient to impede activation and maturation of Th2 cells in susceptible mice, even when targeted directly to the infected cell.

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tracking goals against invention  
for cytosine transfection in general

L9 ANSWER 378 OF 419 MEDLINE  
AN 88309783 MEDLINE  
DN 88309783 PubMed ID: 2841973  
TI **Heterologous** expression of the bifunctional thymidylate synthase-dihydrofolate reductase from **Leishmania major**.  
AU Grumont R; Sirawaraporn W; Santi D V  
CS Department of Biochemistry and Biophysics, University of California, San Francisco 94143.  
NC AI19358 (NIAID)  
SO BIOCHEMISTRY, (1988 May 17) 27 (10) 3776-84.  
Journal code: 0370623. ISSN: 0006-2960.  
CY United States  
DT Journal; Article; (JOURNAL ARTICLE)  
LA English  
FS Priority Journals  
EM 198810  
ED Entered STN: 19900308  
Last Updated on STN: 19980206  
Entered Medline: 19881011  
AB The bifunctional thymidylate synthase-dihydrofolate reductase (TS-DHFR) of **Leishmania major** has been cloned and expressed in *Escherichia coli* and *Saccharomyces cerevisiae*. The strategy involved placing the entire 1560-bp coding sequence into a parent cloning **plasmid** that was designed to permit introduction of unique restriction sites at the 5'- and 3'-ends. In this manner, the entire coding sequence could be easily subcloned into a variety of expression vectors. High levels of TS-DHFR gene expression were driven by tac, pL and T7 RNA pol promoters in *E. coli*, and the GAPDH-ADH-2 promoter in *S. cerevisiae*. *L. major* TS-DHFR also complemented TS deficiency in *E. coli*. In *E. coli*, the protein accumulated to very high levels, but most was present as inactive inclusion bodies. Nevertheless, substantial amounts were soluble; up to 2% of the soluble protein was catalytically active TS-DHFR. In the yeast systems, essentially all of the bifunctional protein was soluble and catalytically active, and crude extracts contained about 100-fold more enzyme than do extracts from wild-type *L. major*. The expressed TS-DHFR from yeast and *E. coli* was purified to homogeneity by methotrexate-Sepharose affinity chromatography. About 8.5 mg of homogeneous, catalytically active protein is obtained from a 1-L culture of yeast, and 1.5 mg was obtained from 1 L of *E. coli* culture. A 200-L fermentation of the yeast expression system yielded a crude extract containing over 4 g of TS-DHFR. (ABSTRACT TRUNCATED AT 250 WORDS)

L9 ANSWER 369 OF 419 MEDLINE  
AN 90083219 MEDLINE  
DN 90083219 PubMed ID: 2594753  
TI **Transfection** of **Leishmania enriettii** and expression of chloramphenicol acetyltransferase gene.  
AU Laban A; Wirth D F  
CS Department of Tropical Public Health, Harvard School of Public Health, Boston, MA 02115.  
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1989 Dec) 86 (23) 9119-23.  
Journal code: 7505876. ISSN: 0027-8424.  
CY United States  
DT Journal; Article; (JOURNAL ARTICLE)  
LA English  
FS Priority Journals  
EM 199001  
ED Entered STN: 19900328  
Last Updated on STN: 19980206  
Entered Medline: 19900119  
AB We report a transient expression **transfection** system in **Leishmania enriettii**. A hybrid gene containing an intergenic region of the alpha-tubulin cluster and the bacterial chloramphenicol

acetyltransferase (CAT; EC 2.3.1.28) gene is expressed after transfection of *L. enriettii* with the hybrid plasmid. The expression of the CAT gene is dependent on the presence of sequences from the alpha-tubulin gene. The hybrid gene is also active in *Leishmania braziliensis* and *Leishmania major*.

L9 ANSWER 368 OF 419 MEDLINE  
AN 90136974 MEDLINE  
DN 90136974 PubMed ID: 2300209  
TI Stable expression of the bacterial neor gene in **Leishmania enriettii**.  
AU Laban A; Tobin J F; Curotto de Lafaille M A; Wirth D F  
CS Department of Tropical Public Health, Harvard School of Public Health, Boston, Massachusetts 02115.  
SO NATURE, (1990 Feb 8) 343 (6258) 572-4.  
Journal code: 0410462. ISSN: 0028-0836.  
CY ENGLAND: United Kingdom  
DT Journal; Article; (JOURNAL ARTICLE)  
LA English  
FS Priority Journals  
EM 199003  
ED Entered STN: 19900328  
Last Updated on STN: 19900328  
Entered Medline: 19900313  
AB Molecular genetic studies in parasitic protozoa have been hindered by the lack of methods for the introduction and expression of modified or foreign genes in these organisms. Two recent reports described the transient expression of the bacterial chloramphenicol acetyl transferase (CAT) gene under the control of parasite-specific sequences. We now describe the stable expression of a selectable marker, the gene for neomycin resistance (neor) in **Leishmania enriettii**. A chimaeric gene containing the neor gene inserted between two alpha-tubulin intergenic sequences was introduced into the cells and drug-resistant *L. enriettii* were observed which stably expressed the neor gene. One goal of this work was to analyse the sequences necessary for trans-splicing of messenger RNA, as trypanosomatids have a novel process of RNA trans-splicing, described initially in *Trypanosome brucei* and subsequently in several other trypanosomatids, including *L. enriettii*. Many trypanosomatid genes are arranged in tandem arrays and the intergenic sequences contain both the splice acceptor site for the addition of the spliced leader sequence and a putative polyadenylation site. Messenger RNA isolated from several different neor *L. enrietti* lines contained the spliced leader sequence joined to the neor gene at the position of the splice acceptor site in the alpha-tubulin intergenic sequence. The neor mRNA was also polyadenylated. Plasmid DNA is present within the drug-resistant organisms and appears to be extrachromosomal. The development of these methods allows the functional analysis of sequences necessary for trans-splicing.

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L9 ANSWER 353 OF 419 MEDLINE  
AN 91304515 MEDLINE  
DN 91304515 PubMed ID: 1906580  
TI Stable DNA **transfection** of a wide range of trypanosomatids.  
AU Coburn C M; Otteman K M; McNeely T; Turco S J; Beverley S M  
CS Department of Biological Chemistry and Molecular Pharmacology, Harvard  
Medical School, Boston, MA 02115.  
NC AI-20941 (NIAID)  
AI-21903 (NIAID)  
GM07196 (NIGMS)  
SO MOLECULAR AND BIOCHEMICAL PARASITOLOGY, (1991 May) 46 (1) 169-79.  
Journal code: 8006324. ISSN: 0166-6851.  
CY Netherlands  
DT Journal; Article; (JOURNAL ARTICLE)  
LA English  
FS Priority Journals  
EM 199108  
ED Entered STN: 19910908  
Last Updated on STN: 19910908  
Entered Medline: 19910821  
AB We have shown that the **Leishmania** major **transfection**  
vector pR-NEO (or derivatives thereof) can be introduced and stably  
maintained in four species complexes of pathogenic **Leishmania**  
(*L. tropica*, *L. mexicana*, *L. donovani*, *L. braziliensis*), and the genera  
*Endotrypanum* and *Crithidia*; **transfection** of *Trypanosoma cruzi* or  
*Trypanosoma brucei* was not successful. Quantitative plating assays showed  
that the **transfection** efficiencies were high in *L. major* and  
*Leishmania amazonensis* ( $5 \times 10^{-5}$ /cell) and about 10-fold less for  
*Leishmania panamaensis* and *Crithidia*. **Leishmania**  
*donovani* **transfected** with pR-NEO retained the ability to infect  
hamsters, and amastigotes recovered after 2 months yielded G418-resistant  
promastigotes which retained high levels of extrachromosomal pR-NEO DNA.  
In promastigotes, the **transfected** DNA existed as  
extrachromosomal circles, and expressed the predicted 2.4-kb hybrid  
NEO/DHFR-TS mRNA bearing the trans-spliced miniexon. Large quantitative  
differences were observed only in *Crithidia*: relative to  
**transfected Leishmania** species, the copy number of  
pR-NEO was elevated 20-fold, while the levels of the NEO/DHFR-TS mRNA or  
*Escherichia coli* beta-galactosidase (synthesized from the expression  
vector pX-beta GAL) were reduced 80 and more than 1000-fold, respectively.  
Thus, genetic signals derived from *L. major* DNA that mediate RNA  
expression or stability are recognized by the **heterologous**  
**Leishmania** species but less efficiently by *Crithidia*. These  
studies suggest that pR-NEO derived vectors may be applied to the study of  
genes expressed throughout the life cycle in a wide range of pathogenic  
trypanosomatids.

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L9 ANSWER 407 OF 419 MEDLINE  
AN 84142075 MEDLINE  
DN 84142075 PubMed ID: 6321982  
TI Isolation and characterization of an alpha-tubulin gene from  
**Leishmania enriettii**.  
AU Wirth D F; Slater C  
SO MOLECULAR AND BIOCHEMICAL PARASITOLOGY, (1983 Sep) 9 (1) 83-92.  
Journal code: 8006324. ISSN: 0166-6851.  
CY Netherlands  
DT Journal; Article; (JOURNAL ARTICLE)  
LA English  
FS Priority Journals  
EM 198404  
ED Entered STN: 19900319  
Last Updated on STN: 19900319  
Entered Medline: 19840417  
AB An alpha-tubulin gene of **Leishmania enriettii** has been identified in genomic Southern blots by hybridization with a heterologous alpha-tubulin gene from *Drosophila melanogaster*. A clone containing this gene has been isolated from a plasmid library of size-selected *L. enriettii* DNA. It was identified by hybridization with the *D. melanogaster* tubulin gene. The cloned DNA fragment was characterized by restriction analysis and partial DNA sequence analysis. The cloned DNA fragment is 2 kb in length, bounded by Pst I sites, and appears to contain the entire coding region of the alpha-tubulin gene.

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